HHS Public Access

Author manuscript

Trends Genet. Author manuscript; available in PMC 2019 April 01.

Published in final edited form as:

Trends Genet. 2018 April ; 34(4): 253–255. doi:10.1016/j.tig.2018.01.009.

A toolkit of engineered recombinational balancers in *C. elegans*

Hillel T Schwartz and **Paul W Sternberg**

Division of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125

Abstract

Dejima and colleagues report using CRISPR-Cas9 to generate a new collection of greatly improved balancer chromosomes in the standard laboratory nematode *Caenorhabditis elegans*, using methods previously reported by the same laboratory [1,2], expanding the set of *C. elegans* balancers to cover nearly 90% of coding genes.

Keywords

Caenorhabditis elegans; CRISPR; Cas9; balancer; recombinational suppression

Text

Balancer chromosomes are a powerful tool in experimental genetics. Balancers are variant chromosomes that when heterozygous with normal chromosomes have a defined region within which there is no or essentially no recombination between the balancer chromosome and the normal chromosome. To achieve this, balancer chromosomes contain rearrangements such as inversions and translocations that disrupt synapsis and so prevent recombination. Any mutations within this recombinationally suppressed region can be reliably followed in *trans* using the balancer. Balancer chromosomes typically carry markers to make this more efficient: dominantly acting markers such as a *gfp* transgene to mark the presence of the balancer, and often a mutation with a recessive visible or even lethal phenotype to mark or to prevent the loss of the non-balancer chromosome and the mutations it contains. Balancers containing such markers can be used to infer genotype rapidly and efficiently.

It is now just over 100 years since the first report of a chromosome acting as a recombinational suppressor, by Sturtevant in 1917 [3]. This result, following close after the discovery that it was possible to construct a genetic linkage map [4], was immediately seen as being consistent with a chromosomal theory of inheritance and was soon shown to respond to chromosomal rearrangements that could be observed cytologically [5]. Much later, work in *C. elegans* with a reciprocal translocation that acted as a recombinational

suppressor [6] was important in showing that although the chromosomes of *C. elegans* are holocentric they nonetheless each have a site, now called the pairing center, that acts genetically like a centrosome, directing pairing and chromosome segregation.

The use of balancers made it possible to identify large numbers of recessive-lethal mutations in *C. elegans*, by screening the progeny of mutagenized balancer heterozygotes to find mutants that were no longer viable as non-balancer homozygotes [7]. In an era of efficient, affordable targeted genomic modification, balancers remain essential in maintaining strains containing mutations with recessive-inviable phenotypes; this report also offers useful guidance in generating more such mutations, when CRISPR activity is likely to otherwise target both copies and produce inviable homozygous mutants. A good collection of balancer chromosomes covering as much as possible of the genome is incredibly useful in strain constructions: even though most mutations can now be genotyped molecularly, the use of balancers to follow loci in *trans* can make strain constructions cheaper and greatly less labor-intensive than is the case using molecular genotyping. Good balancers also make it possible to collect animals homozygous for recessive-inviable mutations, whether it's single animals for close examination or much larger quantities for biochemical analysis, collected through the use of a sorting machine or by using a selective marker.

The set of balancers previously available in *C. elegans* was a patchwork collection of reciprocal translocations, single inversions, and integrated transgenes [8]. This set failed to cover large portions of the genome, and most parts of this set had major inconveniences and liabilities. Translocations are stable but cause pseudolinkage across two chromosomes that can make them less useful; more importantly, translocation heterozygotes generate large numbers of aneuploid embryos that can impede analysis of balanced mutants (10/16 of self-progeny of translocation heterozygotes are aneuploids of one sort or another). Large single inversions break down at high frequency. Transgenes are available that have been randomly integrated at many points in the genome, but tend to be poorly characterized to determine across what regions if any they completely suppress recombination.

The new set of engineered balancer chromosomes offers a significant new resource, addressing a large gap in the tools available to the *C. elegans* research community. The new rationally designed balancer chromosomes have none of the drawbacks detailed above. In contrast to translocations used as balancers, the CRISPR-made rearrangements are contained within a single chromosome, and so do not cause pseudolinkage. As Dejima and co-workers specifically demonstrate, the new balancers do not generate aneuploid embryos. Each of the new balancers contains two overlapping inversions, making them much less susceptible to spontaneous breakdown than older balancers consisting of a single inversion. Unlike arbitrarily selected transgenes randomly integrated into the chromosome, the limits of the recombinationally suppressed regions are precisely known for these new balancers. In addition to generating large, stable chromosomal rearrangements covering large sections of the genome, the authors also provide markers for regions of the genome close to pairing centers, which are recalcitrant to the engineering of recombinational suppressors, and demonstrate a method for using CRISPR to generate heterozygous mutations that would be inviable as homozygotes.

Pioneering work using nucleases with engineered specificity in *C. elegans* was accompanied by demonstrations that it could be used in a nematode species less commonly used in the laboratory [9]. The model demonstrated by this new collection of engineered balancer chromosomes in *C. elegans* is similarly transferable to other organisms, including organisms that have so far been less popular in laboratory research and so lack the genetic toolkits necessary to enable and accelerate research. This should make it more feasible to generate, maintain, and examine inviable mutants in species not previously popular in the laboratory. In particular, the availability of balancer chromosomes could facilitate genetic screens in male-female nematode species, by making it possible to follow and to homozygose mutagenized, balanced sections of the genome.

References

1. Dejima K, et al. An aneuploidy-free and structurally defined balancer chromosome toolkit for *Caenorhabditis elegans*. Cell Rep. 2018; 22:232–241. [PubMed: 29298424]
2. Iwata S, et al. Engineering new balancer chromosomes in *C. elegans* via CRISPR/Cas9. Sci Rep. 2016; 6:33840. [PubMed: 27650892]
3. Sturtevant AH. Genetic factors affecting the strength of linkage in *Drosophila*. Proc Natl Acad Sci USA. 1917; 9:555–558.
4. Sturtevant AH. The linear arrangement of six sex-linked factors in *Drosophila*, as shown by their mode of association. J Exp Zool. 1913; 14:43–59.
5. Sturtevant AH, Dobzhansky T. Inversions in the third chromosome of wild races of *Drosophila pseudoobscura*, and their use in the study of the history of the species. Proc Natl Acad Sci USA. 1936; 22:448–450. [PubMed: 16577723]
6. Rosenbluth RE, Baillie DL. The genetic analysis of a reciprocal translocation, *eT1*(*III; V*), in *Caenorhabditis elegans*. Genetics. 1981; 99:415–428. [PubMed: 6953041]
7. Johnsen RC, Baillie DL. Formaldehyde mutagenesis of the *eT1* balanced region in *Caenorhabditis elegans*: dose-response curve and the analysis of mutational events. Mutat Res. 1988; 201:137–147. [PubMed: 3419443]
8. Edgley, ML., Baillie, DL., Riddle, DL., Rose, AM. The *C. elegans*Research Community. WormBook. WormBook; 2006. Genetic Balancers; p. 1-32.
9. Lo TW. Precise and heritable genome editing in evolutionarily diverse nematodes using TALENs and CRISPR/Cas9 to engineer insertions and deletions. Genetics. 2013; 195:331–348. [PubMed: 23934893]

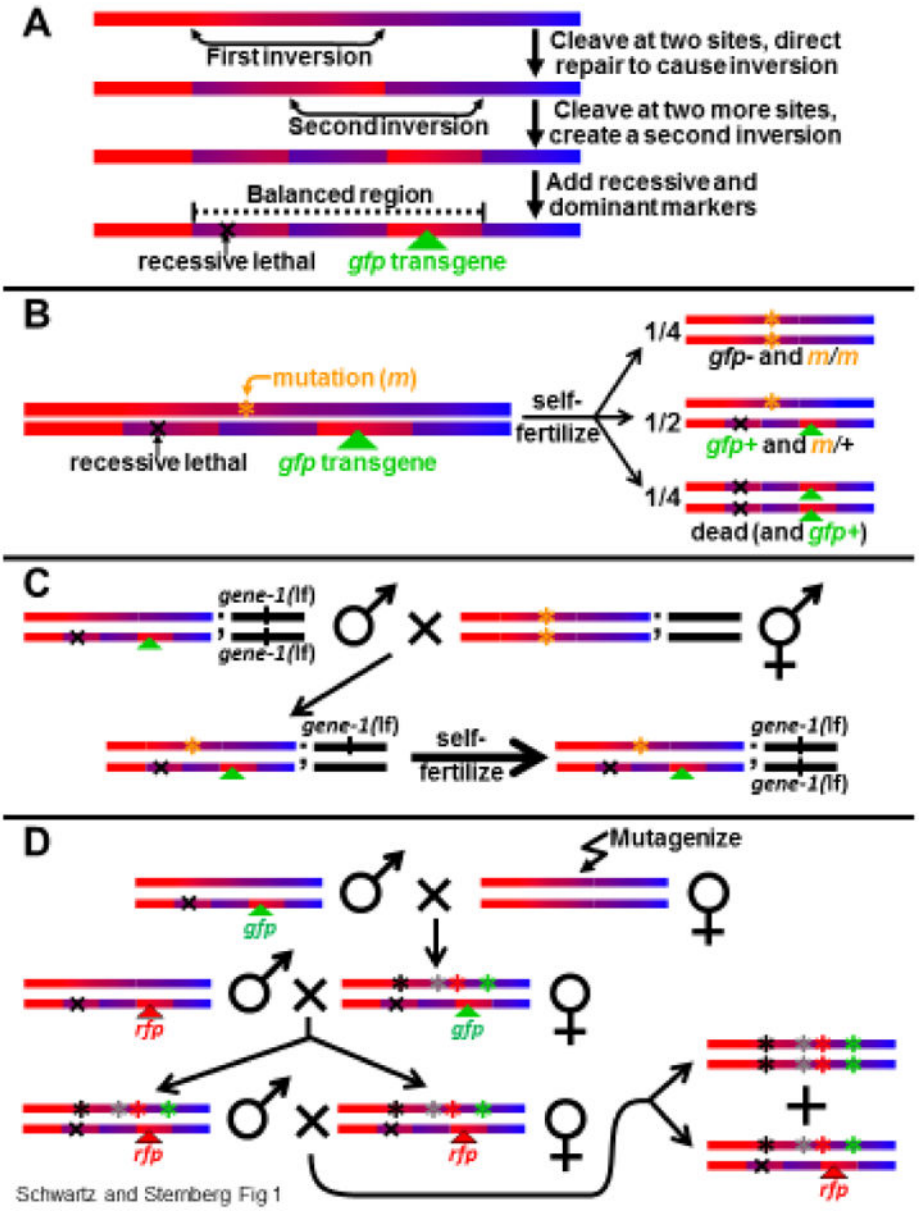

Figure 1.

The generation and use of balancer chromosomes. (A) Balancer chromosomes are engineered using CRISPR. The programmable nuclease Cas9 makes a pair of cuts, excising a large section of a chromosome; the repair of these double-strand DNA breaks is directed by oligonucleotide repair templates to re-integrate that section into the chromosome as an inversion. This process is repeated to create a second, overlapping inversion, to make a more stable recombinational balancer. Markers can then be added into the recombinationally suppressed or “balanced” region of this modified chromosome: dominant markers such as a *gfp* transgene to mark the presence of the balancer in heterozygotes, and recessive markers such as a lethal mutation, to mark or prevent the loss of the non-balancer chromosome. (B) Especially when well marked, balancer chromosomes can be used to maintain strains

carrying mutations with undesirable recessive phenotypes, and to obtain mutation homozygotes from these strains for analysis. (C) By making it possible to follow loci in *trans*, balancer chromosomes can be used to make the construction of double mutant strains more labor-efficient and less dependent on molecular genotyping. (D) Balancer chromosomes can be used to follow sections of the genome following mutagenesis, so that in genetic screens mutants in that region can be examined in homozygotes while also being maintained as heterozygotes. This can make it feasible to conduct genetic screens in male/female species that lack the selfing hermaphroditism of *C. elegans*.